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REVIEW OF LITERATURE

Iwanowsky (9)* first demonstrated that the infective principle of tobacco mosaic disease could pass through filters fine enough to retain bacteria. Due to their amicroscopic size and hydrophylic nature, it has not been possible to measure the diameter of the virus particle by direct microscopic technique. The numerous studies on the particle size of the tobacco mosaic virus have employed three principal techniques—ultrafiltration through graded filters, diffusion, and sedimentation in an ultracentrifuge.

Duggar and Armstrong (3) observed that the virus of tobacco mosaic disease passed through a collodion membrane to about the same extent as a one per cent hemoglobin solution. More recently, Svedberg and Nichols (26) determined the molecular weight of carbon-monoxide hemoglobin and found it to be 68,000 and calculated the molecular diameter to be 5.4 mμ.

Elford (6) developed a method of preparing collodion membranes of uniform pore size, the size of the pores being graded by varying the constituents and method of preparation. Schmith (22) and Thornberry (28) filtered virus solutions through collodion membranes of graded pore size and obtained the values of 15 mμ and 11 mμ respectively as the diameter of the virus particles.

Waugh (31), using the diffusion method of Northrop and Anson (15), determined the diffusion coefficient of tobacco mosaic virus in several purified preparations. He calculated the particle diameter to be 5.62 mμ, which size agrees closely with that found by Duggar and Armstrong (3), considering their results in the light of the recent work on the determination of the size of the hemoglobin molecule.

From data on the sedimentation of the virus protein (23) in an ultracentrifuge, Stanley (24) estimated the molecules to have a molecular weight of 17,000,000. This value greatly exceeds the values reported by any previous workers and is much larger than the molecular weight of most proteins that have been studied. Assuming the virus molecules to have a density of 1.33, a molecular weight of 17,000,000 would correspond to a molecular diameter of approximately 35 mμ.

*See Bibliography, page 17.

Bernal and Fankuchen (2) made X-ray studies of the structure of crystalline virus protein prepared by Stanley's method. The protein molecules were observed to be composed of piles of sub-molecules of dimensions 22 A° x 20 A° x 20 A°, which dimensions are slightly smaller than normal protein molecules.

Bernal (19) observed as a result of further X-ray studies that crystalline virus protein consists of liquid crystals of a globulin type protein. He further states that the virus molecules consist of primary units of 6 x 6 x 6.9 mμ. On the basis of these results it would seem that Stanley's macromolecules probably do not exist as such in the sap of infected plants.

METHOD AND APPARATUS

Theory of the Method

The diffusion coefficient "D" is defined as the quantity of substance that will diffuse across a permeable plane of unit dimensions in unit time under unit concentration gradient or, as given by Nernst (14).

$$dS = -D.q.\frac{dc}{dx}.dz \qquad (I)$$

in which q represents the area, dz the time interval, $\frac{dc}{dx}$ the concentration gradient and dS represents the quantity diffused expressed as cc. of the original solution.

By appropriate substitutions Northrop and Anson (15) reduced this equation to the following:

$$D = K.Q.c.c. \qquad (II)$$

in which D represents the diffusion coefficient of a substance Q.c.c. of which diffused in time t, in days. K represents the diffusion constant of the cell.

The relation between the diffusion coefficient and the radius of the molecule is expressed in the following equation by Einstein (4):

$$D = \frac{RT.}{N} \quad \frac{1}{6\pi rN} \qquad (III)$$

$R = 8.31 \times 10^{7}$ ergs deg.$^{-1}$ mole^{-1}

$N = 6.06 \times 10^{23}$ mole^{-1}

$T = 295°$ Abs. (22°C)

$r =$ radius of particle, including water of hydration.

$\eta =$ viscosity of solvent at 295° Abs. $= .00958$ ergs sec. cm.$^{-1}$ (4)

Substituting the above values in equation III gives:

$$D = \frac{8.3 \times 10^{7} \times 295 \text{ erg deg.}^{-1} \text{ mole}^{-1}}{6.06 \times 10^{23} \times 6 \times 3.14159 \times .00958 \times r \times \text{mole}^{-1} \text{ erg sec. cm.}^{-1}}$$

$$= \frac{2.2328 \times 10^{-13} \text{ cm. sec.}^{-1}}{r} = \frac{1.9291 \times 10^{-8} \text{ cms.}}{r}$$

$$r = \frac{1.9291 \times 10^{-8} \text{ cms.}}{D} \qquad \text{(IV)}$$

Standardization of Cell

The diffusion cell used was the No. 2 cell prepared and described by Waugh (31). It consisted of a thin porous sintered glass disc fused into a Jena glass vessel obtained from Schott and Company, Jena, Germany. The disc was less than one-half millimeter in thickness and free from leaks as tested by blowing air into the cell when immersed in water.

The cell was fastened to a microscope focusing adjustment and rigidly held in a vertical position by attaching to a rigid rod at the side of a constant-temperature water bath. The apparatus was equipped with leveling screws so that the cell could be adjusted with the disc in a horizontal position. This leveling was done by lowering the disc to the surface of some mercury in a shallow dish and adjusting the leveling screws until the disc was horizontal.

The entire apparatus was placed on a concrete floor. The bath was regulated by a thermostat and kept at a temperature of 22° ± .1°C throughout all the diffusion experiments. All apparatus remained at least one hour in the water bath before being used.

Before each diffusion experiment, the cell was cleaned with cleaning solution prepared by dissolving potassium bichromate in concentrated sulfuric acid. The cell was rinsed with distilled water seven or eight times, until entirely free of acid and then rinsed once with the solution to be diffused. The cell was then filled to the stopcock with the diffusion liquid and lowered into a 100 cc. beaker containing 30 cc. of water, so that the disc barely made contact with the water surface. After two hours the cell was assumed to have reached equilibrium and the first beaker was replaced by another 100 cc. beaker containing a measured volume of water, which was to contain the diffusate, and diffusion allowed to proceed for the desired length of time.

The volume of the cell was determined by drying the cell twelve hours in an oven at 110°C. and weighing it empty. It was then filled with distilled water at 31°C. and weighed again. This gave the

weight of distilled water necessary to fill the cell at 31°C. From these data the volume of the cell was calculated to be 41.235 cc.

The Cell Constant

The cell constant K in the equation $D = \frac{K.Qcc}{t}$ was determined by the method used by Northrop and Anson (15), except that 2.0 N. hydrochloric acid was allowed to diffuse for twenty minutes. The diffusate was then titrated with .01014 N. NaOH, using methyl red as indicator, and the quantity Qcc. diffused expressed as cc. of 2.0 N. hydrochloric acid. Each result given in the following table represents the average of four titrations.

TABLE 1.—STANDARDIZATION OF THE DIFFUSION CELL WITH 2.0 N. HYDROCHLORIC ACID.

Period	Volume of .01014N Sodium Hydroxide Used		Corrected Reading*
First 20 minutes	42.16		42.16
Second 20 minutes	41.87		42.09
Third 20 minutes	41.37		41.92
Fourth 20 minutes	41.51		42.17
Fifty 20 minutes	41.00		41.87
		Average	42.04

*A correction was necessary since approximately .42 m.e of Hcl diffused from the cell each twenty minutes.

The average volume of 2.0 N. hydrochloric acid diffused per twenty minute period was $\frac{42.04 \times .01014}{2.0} = .2131$ cc.

The diffusion coefficient D for 2.0 N. hydrochloric acid as extrapolated from the values given in the Handbook of Chemistry and Physics (7) is 2.287 cm.^{2} per day.

$$\text{The cell constant (K)} = \frac{D.t}{Qcc} = \frac{2.287 \times \frac{1200}{86{,}400}}{.2131 \text{ cc.}} = .1491$$

The value of K was redetermined at intervals and was found not to change during the series of experiments.

EXPERIMENTAL

Virus Preparations Used

The virus used in this investigation was that of the mosaic disease of tobacco and classified by Johnson (10) as tobacco virus No. 1. Diseased plants of *Nicotiana Tabacum* (*L*) var. Turkish were grown in pots until they were six to ten weeks old. The tops were then removed, frozen, thawed, and the juice expressed with a hydraulic press. This juice was the starting material for all the preparations used. Redistilled water was used throughout the experiments.

Preparation A.—Expressed juice from diseased plants (*N. Tabacum* var. Turkish) was frozen over night. The juice was then thawed and centrifuged fifteen minutes at 3000 r.p.m. This preparation was deep brown in color but free from large suspended particles. It was quite suitable for diffusion experiments. The pH of this preparation was about 5.15.

Preparation B.—Starting with 500 cc. of preparation A, a mixed phosphate eluate was prepared (30). There was no preliminary clearing with lead acetate. Forty cc. of mixed phosphate was used and the final volume was reduced to 250 cc. To this phosphate eluate was added N/1 acetic acid, dropwise until the solution became turbid. The solution was then centrifuged 15 minutes at 3000 r.p.m. and the precipitate suspended in 20 cc. of N/1 phosphate buffer with a hydrogen ion concentration equal to about pH 5.0 and diluted to 100 cc. with water.

Preparation C.—The starting material was the mixed phosphate eluate (30) from 500 cc. of juice. The final volume of this eluate was 250 cc. and to it was added 200 cc. of 1% aqueous safranin solution. The solution was then mixed and allowed to stand from three to six hours. At the end of this time a dense flocculate had settled out. The supernatant liquid was decanted and the precipitate centrifuged. The precipitate was decomposed with twelve grams of Lloyd's alkaloidal reagent (29) and centrifuged again. The 200 cc. of supernatant liquid was frozen, thawed, centrifuged and extracted twice with N. amyl alcohol, using 150 cc. of the N. amyl alcohol for each extraction. The final solution was quite clear and free of red color. The virus fraction was precipitated with N/1 acetic acid. The precipitate packed well on centrifuging. It was washed once to remove traces of alcohol, and then suspended in 20 cc. of M/3 phosphate buffer with a hydrogen ion concentration equal to about pH 5.0. The solution was mixed thoroughly, centrifuged ten minutes at 3000 r.p.m. and diluted to 100 cc. with water.

Preparation D.—To 45 cc. of preparation C was added 5 cc. of .5 saturated magnesium sulfate in .1 N acetate buffer, with a hydrogen ion concentration equal to about pH 5.0. The latter contained about 25 milligrams of crystalline trypsin per 5 cc. The crystalline trypsin was obtained from Dr. John H. Northrop, Rockefeller Institute, Princeton, New Jersey.

Method of Measuring Virus Concentration

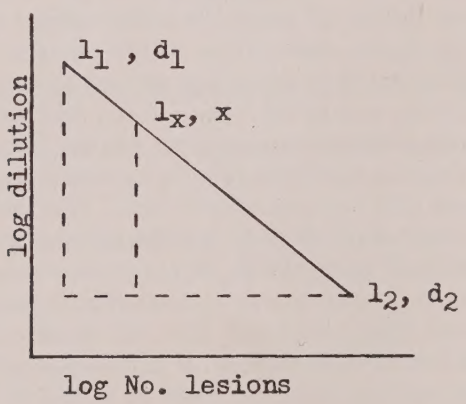
The half leaf method of Samuel and Bald (20) which is a modification of Holmes' (8) local lesion method, was used in measuring virus

concentration. *N. glutinosa* and *Phaseolus vulgaris,* var. Scotia were used as test plants.

The virus concentration of the diffusate from a diffusion experiment was determined by rubbing a cloth, soaked in the solution, once firmly over the right half of about ten leaves. The other half of the same leaves was inoculated with a known dilution of the original preparation used in the diffusion cell. Under side inoculations were avoided by holding a small square of heavy wrapping paper under the leaf.

Local necrotic lesions made their appearance three to four days after inoculation and final counts were made two to six days later. The number of lesions on each half leaf was recorded separately.

Samuel and Bald (20) found that if the logarithm of the number of lesions was plotted against the logarithm of the corresponding dilution, the resulting curve approximated a straight line of slope .60.



Thus in calculating the dilution of each diffusate, two successive dilutions were chosen such that the number of lesions from the diffusate fell between the number of lesions from the two respective dilutions. Then, since the log-log curve is a straight line, it is possible to interpolate between the two known dilutions and obtain the logarithm of the dilution of the diffusate.

Let x be the dilution of the diffusate which produces l_x number of lesions. Let d_1 and d_2 be two successive dilutions whose lesion counts are l_1 and l_2 respectively.

If $l_1 < l_x < l_2$ and $d_1 > d_2$, then by the geometric proportion the following equation results:

$$\frac{\log x - \log d_2}{\log d_1 - \log d_2} = \frac{\log 1_2 - \log 1_x}{\log 1_2 - \log 1_1}$$

$$\text{Then } \log x = \log d_2 + \frac{(\log 1_2 - \log 1_x)\ (\log d_1 - \log d_2)}{\log 1_2 - \log 1_1} \qquad \text{(V)}$$

The same equation (V) may be used for the case in which $1_x > 1_2 > 1_1$.

The antilogarithm of this value gives the dilution and the reciprocal of the dilution is the concentration expressed as a decimal fraction of the original undiluted preparation which was used in the diffusion cell. This concentration multiplied by the volume of the diffusate gives Qcc. in equation (II).

Trypsin-Virus Diffusion

For the virus-trypsin diffusion experiments the cell liquid consisted of one volume of either *Preparation C* or *Preparation A* plus one volume of .5% saturated magnesium sulfate, .1 N acetate buffer solution with a hydrogen ion concentration equal to about pH 5.0 and containing approximately 4 milligrams of crystalline trypsin per cc.

This cell solution was allowed to diffuse for one day into 20.6 cc. of water. The diffusate liquid was the same as the virus-trypsin solution except that one volume of sterile virus preparation was used in place of the infectious preparation. This sterile preparation was prepared by heating an infectious preparation to 93°-96°C. for ten minutes and then centrifuging off the coagulum. Price (18) reported that this temperature was sufficient to completely inactivate the virus. Outside the cell was a solution comparable to the cell solution except that it contained neither trypsin nor virus.

The relative amount of trypsin in the diffusate was determined by the gelatin viscosity method developed by Northrop and Kunitz (17) and later used by Scherp (21) to measure the diffusion coefficient and molecular radius of crystalline trypsin. For the viscosity determinations 500 cc. of isoelectric gelatin was prepared as described by Northrop and Kunitz (17).

The relative particle size of virus particles diffusing from a virus-trypsin solution was also determined. The cell liquid in these experiments consisted of a solution containing 45 cc. of preparation C plus 5 cc. of a .5% saturated magnesium sulfate, .1 N acetate buffer solution with a hydrogen ion concentration equal to about pH 4.0 and containing 25 milligrams of crystalline trypsin. The solution was allowed to diffuse for one day into 20.6 cc. of a solution the same as

that inside the cell, except that it contained neither virus nor trypsin. The diffusate and a portion of the cell liquid, diluted 1:50 were heated to 70°C. for thirty minutes to inactivate the trypsin. The virus concentration of the diffusate was then determined by the half leaf method already described. Adsorption of trypsin by the virus would increase the apparent particle size of the virus and decrease the rate of diffusion, assuming that the number of trypsin particles in solution was equal to or greater than the number of virus particles present, and also assuming that no inactivation of the virus took place in heating the diffusate and cell liquid at 70°C. for 30 minutes or that the same degree of inactivation took place.

Viscosity Measurements

It was considered desirable to investigate the lyophilic properties of some of the purified virus preparations, since these properties may affect the diffusion of the virus particles.

By viscosity measurements at various hydrogen ion concentrations it was possible to determine the isoelectric point and also the relative degree of hydration of the protein particles in these purified virus preparations.

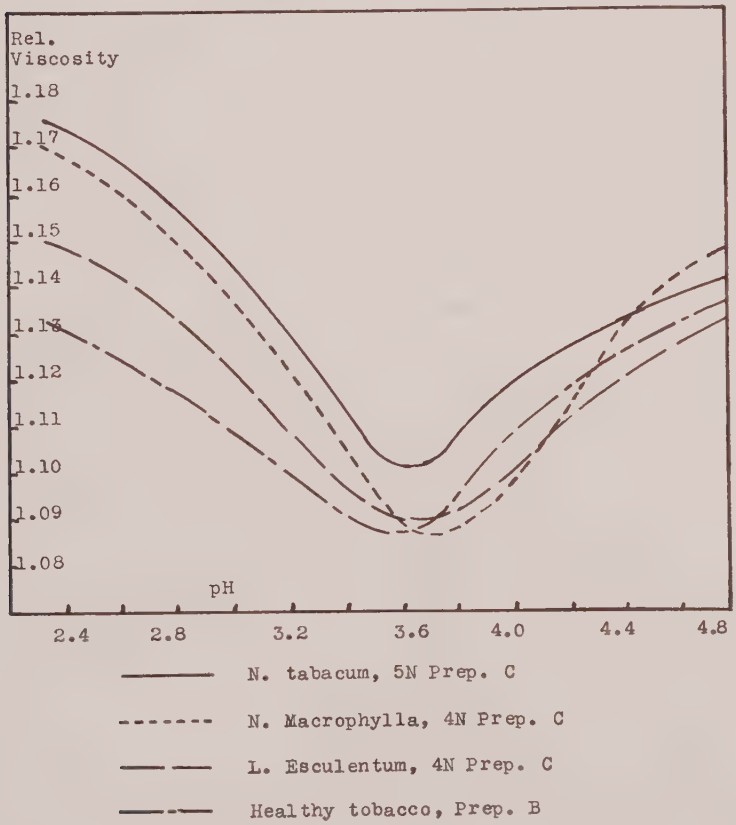
If the protein in these fractions consists of pure virus, or of a definite virus-protein complex, then comparable preparations from different species would be expected to give approximately the same isoelectric point. Likewise, if the virus is present in these plant protein preparations merely as an impurity, then these preparations should have the properties of the plant protein from that particular species and, therefore, should vary in their properties.

Graph 1 shows the viscosity curves for *Preparation C* from juice of diseased *N. Tabacum*, var. Turkish; *N. Macrophylla; Lycopersicum esculentum*, var. Marglobe; and *Preparation B* from juice of *healthy* plants of *N. Tabacum*, var. Turkish. It was not possible to obtain an acid precipitate in *Preparation C* from juice of healthy tobacco plants, thus *Preparation B* was used.

One volume of the virus preparation was added to one volume of N/1 acetate buffer at the desired hydrogen ion concentration. The viscosity was then measured by means of an Ostwald viscosimeter in a water bath at a constant temperature of 22°C. ± .1°C. The hydrogen ion concentration of each solution was measured by means of a quinhydrone electrode.

The isoelectric points of the four preparations used were approximately equal and of the value of pH 3.6 ± .1. In the region of pH

3.2 to 3.9 each of these preparations became slightly cloudy, but flocculation was very slow and did not affect the measurements. The preparation from healthy tobacco plants gave the same type of curve as the preparations from diseased plants, indicating that the same protein may occur in both.



Graph 1.—Viscosity curves of virus preparations from different host plants.

Takahashi and Rawlings (27) were unable to determine the isoelectric point of tobacco mosaic virus by the method of cataphoresis. They found that the virus of tobacco mosaic disease migrated to the anode at pH 4-9 but did not migrate to either electrode at a lower pH. Their results would suggest that the isoelectric point is below pH 4.0. The reason for the virus not migrating to either electrode at pH 3 to 1.2 was not determined.

It may be concluded from the graphs representing the data obtained that the virus of tobacco mosaic disease is closely associated with a protein fraction whose isoelectric point is at a hydrogen ion concentration equal to pH 3.6 ± .1. Furthermore, it would seem that the procedures given by Vinson (29) and Vinson and Petre (30) tend to separate out a protein fraction of definite properties.

The value of pH 3.6 agrees with the value of pH 3.4 ± .1 reported by Best (1). Stanley and Loring (25) determined the pH mobility curve of a solution of virus crystals and found a slightly lower value of the isoelectric point; namely, pH 3.2.

The relation between the relative viscosity of a colloidal solution and the volume of the dispersed phase is given by Einstein's (5) equation, which may be written in the form (11) $\eta s = \eta o \ (1 + \frac{5\phi}{2})$, in which

ηs = viscosity of the solution
ηo = viscosity of the solvent
ϕ = fraction of the volume occupied by the solute.

Arrhenius' equation, as given by Loeb (12) more nearly fits the observed value for protein solutions:

$$\text{Log } \eta s - \log \eta o = K.\phi$$

where ηs, ηo and ϕ have the same meaning as above and K is a constant.

By substituting the values from the curve for diseased *N. Tabacum,* it is calculated that the effective volume of the particles at pH 4.6 is 62.8% greater than at pH 3.7.

This suggests the necessity of performing the diffusion experiments at a uniform hydrogen ion concentration in order to obtain comparable and reliable results.

Effect of Finely Divided Charcoal

The effect of diluting juice from diseased tobacco plants with sterilized juice and the effect of adding charcoal to the inoculum were investigated preliminary to the regular diffusion experiments. The solutions were compared by the half leaf method of inoculation, using as test plants *Phaseolus vulgaris,* var. Scotia.

TABLE 2.—EFFECT OF STERILE JUICE AND OF CHARCOAL ON THE INOCULUM.

Solution	No. half leaves inoculated.	Total No. Lesions.
Prep. A, diluted 1:50 with water	20	217
Prep. A, diluted 1:50 with sterilized juice*	20	85
Prep. A, diluted 1:50 with water plus 25 mgs. charcoal per 10 cc.	10	241
Prep. A, diluted 1:50 with water	10	60

*Sterile juice consisted of juice from diseased plants heated to 93-96° C. for 10 minutes, then centrifuged.

A diffusate liquid prepared by heating and centrifuging the same preparation used inside the diffusion cell might be highly comparable to the cell liquid. Since such a preparation appears, however, to exert a distinctly inhibitory effect on the virus, it was thought best to use redistilled water as the diffusate liquid.

The increase in the number of lesions formed when activated charcoal was added to the inoculum agrees with previous results (29). In making inoculations activated charcoal was added to the inoculum at the rate of 25 milligrams per 10 cc. of solution.

TABLE 3.—LESION COUNTS FROM SCOTIA AND GLUTINOSA TEST PLANTS.

Exp. No.	Test Plant	Prep. Used	Total No. Lesions	No. Half Leaves	Relative values—Lesions per 10 half leaves.
1	Glut.	A	x= 33: 1:50 = 71 x= 16: 1:100= 29 x= 60: 1:300= 21	10 8 8	x= 75 1:100=136.0 1:300= 26.2
2	Scotia	A	x= 174: 1:50 =246 x= 66: 1:100= 76 x= 129: 1:300= 98	15 10 16	x= 80.6 1:100= 91.6 1:300= 61.3
3	Scotia	C	x= 260: 1:25 =382 x= 498: 1:75 =398	21 18	x=277 1:25 =407 1:75 =221
4	Glut.	C	x= 10: 1:25 = 22 x= 27: 1:50 = 29 x= 25: 1:75 = 11	9 16 11	x= 22.9 1:50 = 24.6 1:75 = 10.0
5	Scotia	C	x= 150: 1:25 =167 x= 369: 1:50 =396 x= 385: 1:75 =309	12 25 15	x=257 1:50 =276 1:75 =206
6	Scotia	A	x= 99: 1:50 =132 x= 94: 1:75 =125 x= 77: 1:200= 53	16 15 12	x= 64 1:75 = 85.1 1:200= 44.0
7	Scotia	C	x=1170: 1:50 =1184 x= 793: 1:100=596	15 16	x=780 1:50 =789.3 1:100=586.0
8	Glut.	C	x= 531: 1:50 =673 x= 336: 1:100=288 x= 461: 1:300=189	14 17 17	x=379 1:50 =480 1:100=325
9	Glut.	C	x= 605: 1:50 =548 x= 622: 1:100=384 x= 753: 1:300=164	18 17 18	x=366 1:50 =332 1:100=226
10	Scotia	B	x= 59: 1:50 =101 x= 13: 1:100=105 x= 79: 1:300= 79	17 12 17	x= 46.5 1:300= 46.5
11	Scotia	D	x= 132: 1:50 =272 x= 280: 1:100=350 x= 109: 1:300= 96	20 21 15	x=133 1:100=166 1:300=117
12	Glut.	D	x= 25: 1:50 =249 x= 29: 1:100=129 x= 36: 1:300= 45	19 14 10	x= 36 1:100=160 1:300= 45

Particle Size Determinations

Table 3 gives the results of the diffusion experiments to determine the particle size of the virus of mosaic disease of tobacco. Test solutions A, C, and D were allowed to diffuse for one day into a volume of distilled water (20.6 cc.) equal to one-half the volume of the cell. In experiments 1 and 2, the volume of distilled water was 41.2 cc.

Diffusion for one day into 20.6 cc. of water was equivalent to two days into 41.2 cc. of diffusate liquid.

The number of lesions formed with the diffusate x and the dilution used on the opposite half of each leaf are recorded in Column 4. The diffusate did not give the same number of lesions for each half leaf due to variations in susceptibility of individual leaves. The corresponding pairs of lesion counts in experiment 1, for example, are $x = 16$, $1:100 = 29$ and $x = 60$, $1:300 = 21$. These figures are on the basis of eight half leaves. On the basis of ten half leaves the readings would become $x = 20$, $1:100 = 36.3$; and $x = 75$, $1:300 = 26.2$. These two values of x are then set equal to each other by multiplying the first pair of lesion counts by 75/20, which gives the readings: $x = 75$, $1:100 = 136.1$; and $x = 75$, $1:300 = 26.2$. These relative values are given in the last column in Table 3.

The dilution of the diffusate x was then calculated from the values in Table 3 by using equation (V) and the results are given in Table 4. The reciprocal of the dilution gives the concentration.

TABLE 4.—CALCULATION OF RADIUS OF VIRUS PARTICLE.

Experiment Number	Dilution	Concentration 1/Dilution	Volume Diffused (Qcc.)	Diffusion Coefficient (D)	Radius (r)
1	148.8	.00672	.2769	.04129	4.67
2	141.9	.00705	.2903	.04328	4.46
3	49.96	.02002	.4123	.06147	3.14
4	51.6	.01936	.3989	.05948	3.24
5	55.2	.01812	.3732	.05564	3.47
6	114.6	.00873	.1798	.02681	7.20
7	51.4	.01945	.4008	.05976	3.23
8	76.1	.01314	.2707	.04036	4.78
9	41.9	.02384	.4912	.07324	2.63
10*	300.0	.00333	.0687	.01024	18.11
11*	300.6	.00498	.1027	.01531	12.12
12*	364.0	.00275	.0566	.00844	21.98

*Relative viscosity of solvent = 1.04
Average radius of the virus particle = 4.09 ± .31
Average radius of the virus-trypsin particle = 17.40 ± 1.59

The diffusion coefficient D was calculated by substituting in the equation: $D = \frac{KQcc.}{t}$ (II) in which t = time in days = 1. K = .1491. Qcc. = concentration multiplied by the volume of diffusate (column 4, Table 4, equation (IV)). The average radius of the virus particle, as calculated from the results of experiments 1-9 inclusive, is 4.09 ± .31 Mμ. This is slightly greater than the value of 2.81 Mμ found by Waugh (31) and the value of 2.74 Mμ obtained by interpreting Duggar and Armstrong's (3) results in the light of the recent researches on the particle size of the hemoglobin molecule. The value is somewhat lower, however, than that of 11 Mμ reported by Thornberry (28) and 15 Mμ reported by Schmith (22) for the diameter of the virus

particle. It is interesting to note that the two latter results obtained by the ultrafiltration method are distinctly higher than the values of 2.81 Mμ and 4.04 Mμ obtained by the diffusion method. Stanley's (24) estimate of 17,000,000 as the molecular weight of the virus particle would correspond to a particle diameter of about 35 Mμ.

Trypsin-Virus Adsorption

Experiments 10-12 were performed to determine the effect of trypsin in solution upon the rate of diffusion of the virus particles. Calculations of the particle radius are given in Table 4. The mean value obtained for the virus particle in the presence of trypsin was 17.40 $\pm$ 1.59. This indicates that trypsin influences the particle size of the virus—possibly through adsorption. The fact that the virus-trypsin particles have approximately 77 times the volume of the virus particle when alone suggests that in these experiments the concentration of trypsin particles was probably greater than the concentration of virus particles.

The rates of trypsin diffusion from virus-trypsin and from sterile virus-trypsin solutions were compared. The results are given in Table 5. The trypsin-virus solution consisted of one volume of *Preparation C* or *Preparation A* plus one volume of .5 saturated magnesium sulfate solution in .1 N acetate buffer of hydrogen ion concentration equal to about pH 4.0. The trypsin-sterile virus preparation was the same except that one volume of the sterile preparation inactivated by heating at 93°-96°C. for ten minutes and then centrifuging was substituted for the active virus preparation. A measured quantity of crystalline trypsin was dissolved in .5 saturated ammonium sulfate buffered at pH 4.0 with acetate. Aliquots of this solution were used for both the diffusion runs in one series of experiments. Separate trypsin solutions were prepared for each series. The results given in column 5 represent the change in gelatin viscosity per forty minute interval.

TABLE 5.—RATE OF TRYPSIN DIFFUSION FROM TRYPSIN AND TRYPSIN-VIRUS SOLUTIONS.

Series Number	Solution	Milligrams of Trypsin in 41.2 cc. of Digest	cc. Diffusate per 5 cc. of Gelatin	Change in Viscosity in Seconds			
				Diffusate		Blank Cell 1:50	
A	Prep. C + trypsin	81.16	3 cc.	9.5		22.1	
	Sterilized prep. C + trypsin	81.16	3 cc.		11.47		18.05
B	Prep. C + trypsin	57.06	2 cc.	6.0		13.7	
	Sterilized prep. C + trypsin	57.06	2 cc.		9.23		12.9
			Average	7.75	10.35	17.9	15.48

The results in Table 5 show that 33.6 per cent more trypsin diffused from a solution *free* of virus than from one *containing* virus. The virus trypsin solution was always diffused on the first day of the experiment and the sterile virus-trypsin solution diffused on the following day. In this manner the slow decrease in tryptic activity on standing at room temperature did not affect the results. That there was a slight decrease in tryptic activity is shown by a comparison of the blanks (cell liquid diluted 1:50).

It is concluded from these experiments that the presence of the virus of tobacco mosaic disease does affect the diffusion rate of trypsin, probably due to adsorption.

SUMMARY

The radius of the virus particle of mosaic disease of tobacco in solution at a hydrogen ion concentration equal to about pH 5.0 was found to be 4.09 $\pm$ .31 Mμ.

The apparent radius of the virus in the presence of trypsin, at the relative concentrations used, was found to be 17.40 $\pm$ 1.59. This increased particle size is probably due to adsorption of trypsin by the virus particle.

The diffusion rate of trypsin in solution, free from active virus particles is 33.6 per cent greater than when virus particles are present, at the relative concentration used in this investigation. This also indicates adsorption reactions between trypsin and virus.

The isoelectric point of the protein in highly purified virus preparations from three different species of plants is pH 3.6 $\pm$ .1.

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